Supplementary Information

Full details of the methods

Molecular Biology

Human Kir6.2 (Genbank NM000525 with E23 and I377), and rat SUR1 (Genbank L40624) were used in this study. Site-directed mutagenesis of Kir6.2 was performed using the QuickChange^{TM}XL system (Stratagene, La Jolla, CA, USA). Wild-type and mutant cDNAs were cloned in the pBF vector, and capped mRNA prepared using the mMESSAGE mMACHINE large scale *in vitro* transcription kit (Ambion, Austin, TX, USA).

Oocyte preparation

Female *Xenopus laevis* were anaesthetized with MS222 (2g/l added to the water). One ovary was removed via a mini-laparotomy, the incision sutured and the animal allowed to recover. Once the wound had completely healed, the second ovary was removed in a similar operation and the animal was then killed by decapitation whilst under anaesthesia. Immature stage V-VI oocytes were incubated for 60 min with 1.0 mg/ml collagenase (Sigma, type V) and manually defolliculated. Oocytes were either injected with ~1 ng wild-type or mutant Kir6.2ΔC36 mRNA or coinjected with ~2 ng of SUR1 mRNA and ~0.1 ng wild-type or mutant Kir6.2 mRNA. The final injection volume was 50 nl/oocyte. For each batch of oocytes, all mutations were injected in order to enable direct comparison of their effects.

To simulate the heterozygous state, SUR1 was coexpressed with a 1:1 mixture of wild type and mutant Kir6.2. A potential problem with the use of coinjection of wild-type and

mutant mRNAs to simulate the heterozygous state is that the levels of expression may be different for wild-type and mutant proteins. However, other approaches are prone to different errors and as discussed by Proks et al [1], we believe that simulating the heterozygous state by coinjection of wild-type and mutant subunits is the approach least prone to error. It is also important to point out that coexpression of two mRNAs most closely simulates the situation in the patient's cells (where differences in expression may also occur).

Isolated oocytes were maintained in Barth's solution and studied 1-7 days after injection [2]. All procedures used conformed with UK Home Office regulations and the University of Oxford ethical committee guidelines.

References

1. Proks P, Girard C, Ashcroft FM: Enhanced activation by MgATP contributes significantly to the functional effects of *KCNJ11* mutations causing neonatal diabetes. *Hum Mol Genet* 14:2717-26, 2005.
2. Gribble FM, Ashfield R, Ämmälä C, Ashcroft FM: Properties of cloned ATP-sensitive K-currents expressed in *Xenopus* oocytes. *J Physiol* 498.1:87-98, 1997.